THE CONDITIONS OF ACTIVATION OF UNFERTILIZED STARFISH EGGS BY THE ELECTRIC CURRENT.

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While sensitivity to the electric current seems universal in living matter, its degree varies greatly—apparently in correspondence with the wide variation in general irritability. It is most highly developed in rapidly responding tissues such as muscle and nerve; but it can be shown to exist in supposedly insensitive cells like epidermal cells, which respond to electrical stimulation by increase of conductivity.[1] Specialized sensory receptors (retina, auditory or chemical senses) all respond to the electric current as well as to their own appropriate forms of stimulation. Electrical sensitivity thus appears to be the primary form of sensitivity.[2] In general, as the work of Nernst and his successors has shown, it is intimately connected with polarizability, which is dependent on the presence of diffusion-resisting or semipermeable partitions enclosing or pervading the protoplasmic system. Evidently the electric current acts within the living system by influencing the chemical reactions at its polarizable surfaces, as in electrode action in general; in living protoplasm the surfaces concerned are those of the protoplasmic structures, and especially of the films delimiting or separating the protoplasmic phases.

The directive or stimulating action of the electric current on growth has been demonstrated in a number of cases, although much remains to be done in this field. The unfertilized egg-cell, however, seems usually to be relatively insensitive to the current. The earlier evidence of electrical parthenogenesis in the eggs of marine animals is inconclusive. Schücking claims to have activated starfish eggs by passing the current from two chromic acid

[1] Ebbecke, U., *Arch. ges. Physiol.*, 1922, CXCV., pp. 300, 324.

[2] For a fuller discussion *cf.* the recent volume of R. S. Lillie, "Protoplasmic Action and Nervous Action," University of Chicago Press, 1923, pp. 273 *et seq.*

elements for one to two minutes;[1] but the conditions (nature of electrodes, distance apart, quantity of sea-water, etc.) are not described. He does not consider the possibility that acid or alkali electrolytically produced, or heat, rather than the electric current as such, may have been responsible for the effect; and in the light of our own experiments it seems highly probable that this was the case. Schücking found induction shocks to be ineffective, and we have repeated and confirmed this observation. In the condenser-like arrangement used by Delage,[2] the actual physical conditions were ill-defined. Delage's aim was to affect the eggs electrostatically (by induction); they were placed in a layer of sea-water separated by a thin sheet of mica from a sheet of tinfoil, the sea-water and the metal being connected with the poles of a battery. His results were irregular, and he himself expresses doubt as to the real nature of the conditions. The presence of currents and of products of electrolysis seems not to have been excluded.

In the eggs of amphibia McClendon was able to start cleavage and development by passing the alternating current from the lighting circuit (110 volts, 60 cycles) through the water containing the eggs.[3] In this case a current of considerable intensity acted for a brief period (1 to 2 seconds), and the effect was probably not caused by electrolytic products or heat. Further and more precise investigation of the conditions of electrical parthenogenesis in these eggs seems desirable.

Our aim in the experiments described in this paper has been (1) to ascertain whether in fact activation of starfish eggs by the direct electric current is possible, and to what degree, and (2) to determine more precisely the conditions, more particularly of current intensity, time of exposure and temperature, under which the effect is produced. The experiments were performed on the unfertilized eggs of *Asterias forbesii* at Woods Hole during the summers of 1922 and 1923. The direct current was used in all cases. Usually the eggs were exposed to the current during the interval between the breakdown of the germinal vesicle and the separation of the first polar body (prematuration period);[4] for

[1] Schücking, A., *Arch. ges. Physiol.*, 1903, XCVII., 86.

[2] Delage, Y., *Arch. zool. expér. et gén.*, Sér. 4, 1908, IX., p. xxx.

[3] McClendon, J. F., *Amer. Jour. Physiol.*, 1912, XXIX., p. 299.

[4] The period most favorable for fertilization and artificial activation.

comparison a number of observations were made with fully mature eggs. The procedure was simple; the current was passed through a shallow layer of sea-water containing the eggs, and at regular intervals portions of eggs were transferred to dishes or watch-glasses containing sea-water. Afterwards they were examined. Fertilized and unfertilized controls were kept in all cases.

Our preliminary experiments with battery currents of moderate intensity (one to twelve storage cells) gave uniformly negative results, and in all of our later experiments we used the current from the direct current generator of the laboratory. We were not able at first to find suitable electrodes. The ordinary forms of non-polarizable electrodes ("boot" electrodes) proved unsatisfactory because of high resistance and the diffusion of $ZnSO_4$ into the sea-water. When platinum electrodes were used the eggs showed partial activation (membrane-formation) in some experiments; but it could be shown (by first passing the current through a layer of sea-water and then placing the eggs in the sea-water without the current) that this effect was due to—or at least could be produced by—the products of electrolysis. These results indicated the need for an arrangement by which strong currents could be passed through the layer of sea-water without contaminating the latter by the electrode solution or products of electrolysis; and after a number of preliminary experiments the following method was devised. The wires from the direct current line (110 volts) were connected with two broad zinc plates each immersed in a dish of saturated $ZnSO_4$; these dishes with the plates constituted non-polarizable electrodes of low resistance. A rectangular glass vessel containing the sea-water in which the eggs were to be exposed was placed between the dishes. In the experiments of each summer a single vessel was used throughout the whole series; the dimensions in 1922 were 14 x 5.5 x 3.5 cm., in 1923, 13.1 x 6.6 x 3.5 cm. The depth of sea-water was kept constant as nearly as possible throughout each series, 0.4 cm. in 1922 and 0.5 cm. in 1923; the sectional area (from which the estimates of current density were made) was thus 2.2 sq. cm. in 1922 and 3.3 sq. cm. in 1923. The current was conveyed through the sea-water by massive bridges of agar jelly of the same width

as the rectangular vessel and connecting the latter with the electrode dishes. These bridges were made as follows: A concentrated solution of agar-agar in sea-water was allowed to solidify in a large beaker; the mass of jelly was then removed and cut into two blocks of the general shape indicated in the figure; one end of each block stood in the $ZnSO_4$ solution and the other in the sea-water, as shown diagrammatically in the longitudinal section (Fig. 1). By means of this arrangement strong currents (up to 2

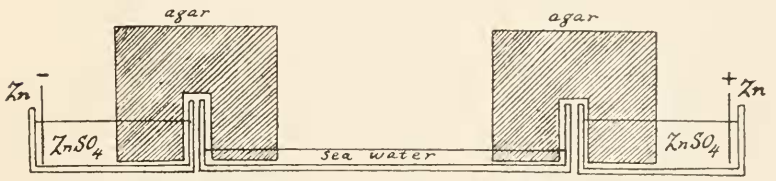

Fig. 1.

or more amperes) could be passed through the sea-water. Experiment showed that the composition of the sea-water was not appreciably affected during the flow of the current for the period of an experiment. The temperature, however, rose rapidly unless controlled. In part of our experiments the control of temperature was effected by setting the whole system in a pan of ice water; this method proved satisfactory with currents of moderate intensity (up to 150 ma./cm.).[1] In a number of experiments with stronger currents another method was used, to be described below. The strength of the current was regulated by two rheostats and measured directly by a Weston voltmeter provided with shunts so as to read as a milliammeter over the several ranges required. Intensities as high as 3 or 4 amperes were used for a brief period in some of the experiments with running sea-water described below (densities up to *ca.* 800 ma./cm.).

The usual procedure was as follows: A somewhat small quantity of eggs was placed with a pipette in the rectangular dish midway between the agar bridges (2 to 4 cm. from each). When the eggs had settled the circuit was closed, and at stated intervals, usually 2, 4, 8 and 12 minutes, successive portions of eggs were transferred to small dishes (usually Syracuse watch glasses or stender dishes)

[1] Milliamperes per square centimeter of sectional area. The commonly used unit of current-density, δ (microampere, *i.e.*, .001 milliampere, per square millimeter), is one tenth of this unit.

containing sea-water; these were kept covered except at times of examination, and the sea-water was changed several times. With small numbers of eggs this method is satisfactory and convenient. In each experiment the temperature of the sea-water in the rectangular dish was recorded at the end of the longest exposure. The temperature was measured by a thermometer with the bulb placed near the eggs. A difficulty with this method is that the bulb was incompletely immersed when the layer of sea-water was shallow, as in most of our experiments; this was especially true of the earlier experiments, where the readings were too low and a correction of 1 to 3 degrees was found necessary; with later experiments a small thermometer with a short bulb was used which gave reliable readings.

A large number of experiments (more than 50) were performed in which the vessel containing the eggs was immersed in ice water as described above; the current densities used ranged from less than 100 to 318 ma./cm. The general results of these experiments may be summarized as follows.

With currents of densities ranging from 136 to 242 ma./cm., flowing from 2 to 12 minutes, activation was either absent or negligible, provided the temperature remained below 29°. In all cases where the temperature rose to 30° or higher a variable degree of activation, usually incomplete, was obtained; and in some cases a considerable proportion of eggs developed to the swimming blastula stage. In the experiment showing the most striking effect of this kind (Aug. 22, 1922) a very typical picture of heat activation was presented; after 4 minutes exposure to a current of 227 ma./cm., only a few eggs (*ca.* 3 per cent.) formed membranes; with exposures of 8 and 12 minutes almost all formed membranes and a large proportion formed blastulæ (*ca.* 75 per cent. with 8 minutes and 25 per cent. with 12 minutes). The temperature at the end of the maximum period of exposure (12 m.), allowing for the error of measurement, was 30° or over. In such a case the activation caused by the current is mainly if not entirely an effect of the high temperature, and not of the current as such. This is shown by the fact that activation was never produced by the same current at lower temperatures (28° or ower); also by control experiments in which eggs were activated

by sea-water which had been warmed to 30° or higher by the current, the latter being shut off before the eggs were introduced.

It seems probable, nevertheless, that a part of the activating effect observed in this and similar experiments is to be attributed to the current; *i.e.*, that there is a summation of the effects of heat and current, since the degree of activation was greater than would usually be produced by exposure to a temperature of 30° for the periods used. According to earlier observations, activation by warm sea-water (acting alone) requires a temperature of at least 29°, and at 30° few eggs develop to a blastula stage after less than 15 minutes' exposure.[1] In other words, the effect of high temperature appears to be greater when a current is flowing through the sea-water containing the eggs than when no current is flowing. We have not, however, performed definite controlled experiments to determine with exactitude the degree of this additive effect. An analogous phenomenon is seen in the activation of starfish eggs by fatty acid; at temperatures of 26° and higher the effective times of exposure to the acid are much shorter than can be accounted for by the temperature cœfficient of acid activation (Q_{10} = *ca.* 3.0) shown at lower temperatures.[2] Apparently in warm sea-water the action of the fatty acid is accelerated by some condition dependent on temperature; *i.e.*, there is a superposition of acid activation upon an incipient heat activation. Similarly, in the experiments with strong currents an effect resulting from the action of the current as such appears to be superposed upon that of the high temperature.

With current-densities higher than 240 ma./cm. the difficulty of evading the temperature effect was such that it was necessary to devise another means of compensating the heating action of the current. We therefore tried exposing the eggs to strong currents in *running* instead of stationary sea-water, and after some preliminary experimentation adopted the following procedure: In place of the rectangular glass dish a paper box of the same dimensions was used. This was reinforced and made an electric non-conductor by several coatings of paraffin. A rectangular slit (*ca.* 6 x 1.5 cm.) was cut at the base of this box along one of the longer sides. A small rectangular cloth basket (*ca.* 3 x 3 x 4 cm.),

[1] Lillie, R. S., Biol. Bull., 1915, XXVIII., p. 260; *cf.* Table II., p. 269.

[2] Lillie, R. S., Biol. Bull., 1917, XXXII., p. 131; *cf.* Table II., p. 142.

containing the eggs, was inserted between the two agar bridges at the center of the paper-paraffin dish. This basket consisted of a frame of slender wooden sticks reinforced with thread and paraffin, to which silk bolting cloth was sewn. The bolting cloth had 200 threads to the inch—thus confining the eggs, yet allowing a free circulation of water. The apparatus was set on a wooden block in a large pan. A stream of sea water of considerable force was directed through a glass nozzle against the bottom of the paper-paraffin vessel on the opposite side of the basket from the slit. In this way a swift stream of water through the basket was obtained. After passing through the basket the water ran out of the slit down the side of the block into the pan where it was removed with a siphon. A small thermometer was p'aced with its bulb resting on the bottom of the basket.

In carrying out these experiments the usual procedure was as follows. The sea water and the current were started and the temperature was allowed to reach its position of equilibrium. Then with a pipette a quantity of eggs was placed in the cloth basket, and at the stated intervals the portions were removed to stender dishes of sea water for observation.

The density of current was estimated from the ammeter reading and the average depth of the layer of running water in the paraffined vessel. Under these conditions, even with densities so high as 600 to 800 ma./cm., the current could be passed for several minutes without raising the temperature above 29°, and with lower densities the temperature showed little increase over that of the sea water without the current. In one of the experiments with a strong current (ammeter reading 680–790 ma./cm.) passed for four minutes, the temperature reading was 29° for most of the period of flow but reached 30°–31° for a few seconds toward the end. In a second similar experiment, with a range of 648–810 ma./cm., in which eggs were removed to normal sea water at intervals of $\frac{1}{2}$, 1, $1\frac{1}{2}$ and 2 minutes, the temperature reading was unfortunately lost; probably 30°–31° was reached in this case also.

With such strong currents the eggs showed marked deformation during the period of exposure, adopting shapes of the kind shown in Fig. 2. This effect is temporary; within a few minutes after

removal from the current the eggs return to their original shape. Such eggs show a separation of fertilization-membranes in a considerable proportion of cases, but not in all. In the experiment just cited *ca.* 40 per cent. of the eggs exposed to the strong

FIG. 2.

current for $1\frac{1}{2}$ and 2 minutes showed well separated membranes and a small proportion developed to the blastula stage. The majority underwent disintegration inside the membrane without development.

Of twelve experiments performed with this method (with good controls), using currents of densities ranging between 130 and 810 ma./cm., seven showed a varying degree of activation. Little or no activation was obtained with currents of less than 300 ma./cm. With higher intensities membrane-formation and activation occurred in a minority of eggs; the strongest currents in addition to deforming the eggs temporarily in the manner just described had a marked destructive effect.[1]

In general the results of the foregoing experiments indicate that the electric current has little activating effect upon the starfish egg unless the intensities employed are sufficient to produce well marked structural changes in the egg-system. The evidence of these changes is deformation and subsequent breakdown of most eggs. A certain proportion of eggs, however, recover and show the usual phenomena of partial activation. These effects cannot be referred to the observed rise of temperature which produces no such definite deformation. Moreover, the highest temperatures reached (30°–31°) require a much longer period of exposure for activation of the degree observed.

It should be remembered in considering the results of such experiments that the physical conditions are far from constant, and that the records of both temperature and current are subject

[1] This destructive action of strong currents on egg cells has been noted by other observers; *cf.* the case of *Crepidula* as described by Conklin, *Jour. Acad. Nat. Sciences*, Philadelphia, 1912, XV., p. 521.

to an error which may be considerable. In general the recorded current density is greater than that to which the eggs were actually exposed, since the current lines extend outside of the rectangular vessel into the overflow. There is also an error of measurement resulting from variability in the depth and contour of the layer of water between the agar bridges; this condition makes possible only an approximate estimate of the sectional areas. The usual procedure was to measure the depth of the water in the basket and on both sides of it and to calculate the cross section from these measurements. But even during a single 5-minute experiment the water level often varied considerably, sometimes because the eggs themselves clogged the silk bolting cloth thus raising the level of water inside the basket. Again, with regard to temperature, although the bulb of the thermometer was completely immersed the irregularity of the water stream is a source of uncertainty. Local temperatures may rise higher than the average temperature recorded by the thermometer; or the instrument may be actually registering the temperature of a stream of water of greater or less velocity than that to which the eggs are actually exposed. It may reasonably be assumed, however, that on the whole the errors in opposite directions compensate each other.

It was thought possible that the physiological effect of the current might be changed (increased or decreased) by changing the balance of salts in the medium. This occurs, for example, in the electrical stimulation of muscle.[1] Eggs were suspended in pure isotonic NaCl solution (0.54 *m*), washed in this solution by gentle centrifuging and decantation, and exposed to the current (densities from 139 to 262 ma./cm.) in the rectangular glass dishes under the conditions already described. A certain degree of activation results from the action of the pure NaCl solution in the absence of the current.[2] The result of passing the current through the NaCl solution containing the eggs was, however, essentially negative; four out of eleven experiments showed a slight increase in activation over that produced by the solution alone, five showed no difference, while two showed a decrease.

[1] *Cf.* the observations of K. Lucas and G. W. Mines on the electrical stimulation of muscle in *Journ. Physiol.*, 1908, XXXVII., p. 459.

[2] Lillie, R. S., *Amer. Journ. Physiol.*, 1911, XXVII., p. 289.

The combined action of the NaCl solution and the current thus shows no significant difference from that of the pure solution alone. The increase noted in the first four experiments was probably the result of a slight rise of temperature; this increases the activating effect of the NaCl solution, as control experiments showed.

Decreasing the conductivity of the medium by adding isotonic sugar solution to the sea water was also found not to alter the effect of the current on the eggs.

In conclusion brief mention should be made of similar experiments with *Arbacia* eggs. The results of these experiments were mainly negative. Little or no effect was produced by exposing the eggs in standing sea water to current-densities varying from 7.5 to 210 ma./cm. No membranes were formed and no cleavage resulted. Eggs exposed to the current and immediately afterwards treated with hypertonic sea water showed no constant increase in the percentage of activation, above eggs treated with hypertonic sea water alone. The effect of exposing to currents of high density in running sea water was also essentially negative, although some cytolysis was caused by the longer exposures. In general the *Arbacia* egg is more resistant to the current than the *Asterias* egg; this difference is probably to be correlated with the greater impermeability of the surface layer to water [1] (and presumably to water soluble substances) and its greater resistance to alteration and the action of parthenogenetic agents in general.

Summary.

1. A new type of non-polarizable ($Zn-ZnSO_4$) electrode of low resistance is described by which strong electric currents (up to 2 amperes or more) can be passed for prolonged periods through a small quantity of sea-water without appreciably affecting its composition.

2. It was found that unfertilized starfish eggs can be readily and completely activated by moderate currents, of the density 200–300 milliamperes per square centimeter; but that the effect in such cases is due almost entirely to the heating action of the current on the sea-water. When the temperature is kept below 29° such currents produce little or no effect upon the eggs.

[1] Lillie, R. S., *Amer. Journ. Physiol.*, 1918, XLV., p. 406; *cf.* p. 420.

Unfertilized eggs are thus insensitive to the current as compared with most irritable or active cells of other kinds.

3. When the eggs are exposed in a stream of running sea-water to stronger currents (600–800 ma./cm.) for brief periods ($\frac{1}{2}$ to 2 minutes), definite effects are produced. The eggs undergo marked deformation during the passage of the current, and a variable proportion afterwards show fertilization-membranes and partial activation. Complete activation of a large proportion of eggs was not possible in our experiments, although a few developed to the blastula stage.

4. We conclude that the unfertilized starfish egg is insensitive to currents of moderate intensity, and exhibits activation only when currents are used of such intensity as to produce definite and well marked structural changes in the egg-system.

5. Similar exposure of unfertilized sea-urchin eggs (*Arbacia*) to strong currents, with and without after-treatment with hypertonic sea-water, gave inconstant or negative results.